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Bacterial Counts in the Muskegon Area Watershed

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Winogradsky Column Bacteria Samples

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Background information:

Sergei Winogradsky developed a method to study how microorganisms live and flourish in a natural setting in a lake or stream. Known as the Winogradsky column, components of microbial ecology of the soil and water can be studied in a variety of ways. These include studying the change in sulfur, nitrogen, carbon, phosphorus, and other nutrients. The Winogradsky column is especially effective because the variables controlled in nature, such as light and temperature, can be controlled by the scientist, and therefore allows for little experimental error (Pryfogle).

Whether or not sunlight affects bacterial growth depends on the bacteria. Few bacteria require sunlight. In dormancy, Anthrax can exist without sunlight for decades. On the other hand, Purple Sulfur Bacteria is a photosynthetic bacteria that requires sunlight in order to reduce carbon dioxide into carbohydrate (Alonso-Sáez). Most bacteria's ideal state for growth is in a damp and dark place where the ultraviolet light from sun cannot degrade the DNA and inhibit bacterial growth (Boundless).

Motivation:

Our motivation for this experiment was a curiosity for the microbial communities living within the soils around the Holland area. We decided to look into these microbial communities in light of the dangerous bacterial growths in other Great Lakes regions.

Our Research:

The aim of this research was to begin looking at the different types of bacteria present in each location with the use of Winogradsky columns. Bacteria counts were taken from the non-sulfur region of each column in order to analyze the effect of sunlight and the addition of calcium carbonate. While this data has no specific implications, it will provide a base for further in depth studies of the bacterial growth in these regions.

Methods:

The Winogradsky columns were prepared by collecting soil and water from four different locations: the Kalamazoo River, Gilligan Lake, Lake Macatawa, and Flower Creek Dunes. For the Kalamazoo River, Gilligan Lake, and Lake Macatawa only one site was sampled. At the Flower Creek Dunes collections were taken from three different sites, the shore of Lake Michigan, the mid-dune, and the forest. Each site was used to prepare two columns, one with an eggshell and one without. Over the course of 16 weeks, data was gathered on the columns by observing the smell and appearance of the bacteria growth. In the 16th week, a sample of bacteria was taken from the non-sulfur bacteria section of each column. One gram of each sample was added to a vial and diluted with saline to create a 10% dilution. This dilution was mixed and then used to swab a petri dish. Six replications were completed for each location. The petri dishes were stored for a week. Using a 1cmx1cm grid, two squares were randomly selected and the number of bacteria in the square was recorded. This data was then analyzed to determine whether sunlight or the calcium carbonate in egg shell has an affect on bacteria.

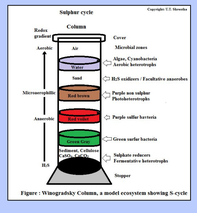


Figure : Winogradsky Column, a model ecosystem showing S cycle

Research questions:

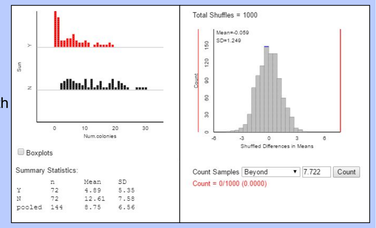
- Is there diverse bacterial growth between locations in the Holland area?
- Does sunlight affect the bacterial growth in these areas?
- Will a mineral deposit such as the calcium carbonate found in an eggshell affect bacterial growth?

Bacteria in Sun Versus Shade Conditions

Comparing the bacteria colonies that grew in sunlit conditions versus shaded conditions.

Hypotheses:

- Null hypothesis (H_0): There is no association between sunlight and bacterial growth in the winogradsky columns. ($\mu_{shade} - \mu_{sun} = 0$).
- Alternative hypothesis (H_a): There is an association between sunlight and bacterial growth in winogradsky columns. ($\mu_{shade} - \mu_{sun} \neq 0$).



Simulation-based inferences:

Bacteria Counts by Sun vs. No Sun

Differences in Means: 7.722 P-value: 0

95% Confidence Interval:

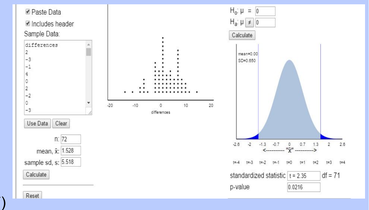
N - Y: (5.56, 9.88)

Bacteria with Egg Shell Versus No Egg Shell

Comparing difference of bacterial growth in the columns containing an egg shell versus no egg shell.

Hypotheses:

- Null hypothesis: there is no difference in the mean colonies of bacteria in the egg shell condition and the non-egg shell condition.
- Alternative hypothesis: there is a difference in the mean colonies of bacteria in the egg shell condition and the non-egg shell.



Theory Based Test for Single Mean:

Bacteria Counts Shell vs No Shell

P-value: .0216

95% Confidence Interval:

Mean Difference (No Shell-Shell):(0.2313, 2.8247)

Bacterial growth by Location

Comparing difference in bacterial growth in each location. Note: we separated the statistical analysis of columns based on whether or not the were in the sunlight or shade conditions to remove this as a confounding variable.

Hypotheses:

- Null hypothesis: there is no difference between each location and the amount of bacterial growth within light and shell conditions.
- Alternative hypothesis: there is a difference between each location and the amount of bacterial growth within light and shell conditions.

Theory-based inferences:

Bacteria Counts By Location (Sun and No Shell)

MAD: 8.611 P-Value: 0

95% Confidence Interval:

GilliganLake - Forest: (-15.80, -10.65)

LakeMI - GilliganLake: (2.32, 6.85)

LakeMI - Forest: (-10.60, -6.07)

Bacteria Counts By Location (Sun and Shell)

MAD: 3.778 P-Value: 0

95% Confidence Interval:

GilliganLakeSh-MidDuneSh: (-6.34, -4.16)

GilliganLakeSh-LakeMISh: (-0.674, 1.507)

LakeMichSh - MidDuneSh: (-6.76, -4.58)

Bacteria Counts by Location (Shade and No Shell)

MAD: 8.722 P-Value: 0

95% Confidence Intervals

KzooRiver - MidDune: (0.96, 8.04)

MidDune - LakeMacatawa: (-16.30, -9.54)

KzooRiver - LakeMacatawa: (-12.13, -5.04)

Bacteria Counts by Location (Shade and Shell)

MAD: 11.556 P-Value: 0

95% Confidence Intervals

LakeMacatawaSh-KzooRiverSh: (14.48, 20.19)

LakeMacatawaSh-ForestSh: (1.48, 7.19)

KzooRiverSh-ForestSh: (-15.85, -10.50)

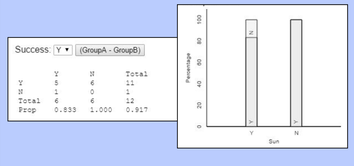
Bacteria Growth on the Water Surface

Comparing the difference in bacterial growth on the water surface in columns in sunlit conditions and shade conditions.

Crust on water (Sun vs. No Sun)

Difference in Proportions (Sun - No Sun): -0.167

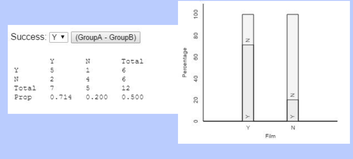
P-Value: 1.000



Film on water (Sun vs. No Sun)

Difference in Proportions (Sun - No Sun): .514

P-Value: .2570



Results:

The p-value of zero in our shade versus sun significance test shows there is strong evidence of a difference between sunlight and the growth of bacteria in the regions in which we collected samples. The confidence interval tells us that the long run average number of colonies is higher in non-sun environments by between 5.56 and 9.88 colonies.This shows non-sun environments similar to the areas we tested have significantly more of the nonsulfur bacteria colonies than sunny environments.

The p-value of .0216 in our shell versus no shell significance test shows there is strong evidence of a difference in the columns containing shell and those not containing shell. The confidence interval shows that the long run average number of colonies is higher in the no shell condition by .2313 to 2.8247 colonies than the shell condition. This shows that calcium carbonate deposits hinder bacterial growth.

Since there was a significant difference between sun and shade as well as shell and no shell, analysis on the difference between locations was split into four categories to account for confounding variable: sun and no shell, sun and shell, shade and no shell, and shade and shell. All of these conditions produced a p-value of 0 indicating strong evidence of a difference in the three locations contained within the condition. The confidence intervals showed that all three locations had significantly different bacterial growth than each of the others for the sun and no shell, shade and shell, and shade and no shell conditions. However, the confidence interval for the sun and shell condition showed that there was a significant difference in bacterial growth between Gilligan Lake shell and the Mid Dune shell and between Lake Michigan shell and Mid Dune shell, but not between Gilligan Lake shell and Lake Michigan shell.

Discussion:

There a a few limitations with the this study. Samples were not collected from a variety of sites for each condition, therefore, they can not be widely generalized. Also, the only bacteria count taken was from the middle of the Winogradsky columns. This leaves certain types of bacteria uncounted. In order to generalize the data collected, samples should have been taken from multiple sites at each location and bacterial growth in each section of the Winogradsky column analyzed.

While these results hold no specific implications they raise a few questions. Adding calcium carbonate from the eggshell to the sample decreased the bacterial counts, but why this occurred is unknown. There did not appear to be any other studies looking into this either. Another question is why adding the eggshell made the bacterial growth of Gilligan Lake and Lake Michigan significantly similar, while without eggshell they were significantly different. This study also did not identify the bacteria that was counted or the effects of this bacteria on the locations it was found in.

References:

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